Toxicological Evaluation of Exposure to Two Formulation of a Pyrotechnically-Generated Aerosol: Range Finding and Multiple Dose

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ABSTRACT: Fischer 344 rats (250-300 g) were exposed to the by-products of two formulations of SFE Formulation **A,** a pyrotechnically-generated aerosol fire extinguishant. Exposure levels ranged from 50 g/m^3 (nominal concentration) to 240 g/m^3. The length of exposure was either 15 or 60 minutes under static conditions. A 700 L whole-body inhalation chamber was used for the exposures and consisted of a supply/exhaust system, aerosol generator and exhaust scrubber. The chamber atmosphere underwent aerosol [size (MMAD), distribution (og) and concentration] and gas [CO, CO, and O_2] analysis. Clinical observations were taken throughout the exposure. Animals were euthanized 1-hr post-exposure and underwent histopathological examination and blood gas analysis. The difference in formulation caused dramatic results in survivability. The first formulation produced levels of carbon monoxide very close to lethal concentrations, while the second formulation showed little if any production. The lack of carbon monoxide production during pyrolyzation is the key difference in the survivability and toxicity of the two formulations.

Introduction

Spectrex Fire Extinguishant (SFE) is a dry powder, soluble, aerosol fire suppressant that is pyrotechnically generated. At temperatures of >500°C, the parent material pyrolyzes to a finely dispersed, optically dense, aerosol cloud. Dry powder extinguishants derive their suppression capabilities from chemical mechanisms and physical properties such as: vaporization, decrepidation, decomposition, surface mediated phenomena, mass concentration, size distribution, specific surface area, density, morphometry, dynamic behavior and chemical composition. These same mechanisms and properties are basic determinants of aerosol inhalation toxicity.

Chemical and physical properties of inhaled particulate matter must be examined simultaneously when studying aerosol toxicity. The dose to regional lung tissue is dependent on particle size, specific surface area and chemical solubility. Therefore, the potential toxic effects from exposure to soluble aerosols may include altered pulmonary function, irritation and altered gas exchange; resulting in discomfort, incapacitation and possible death.

To evaluate the toxicity of an aerosol, it must first be determined if the aerosol can be inspired. The aerosol by-product of SFE has a mass medium aerodynamic diameter (MMAD) of approximately **3** pm. Particles with an MMAD of 3 μm can penetrate deep into the tracheobronchial tree, reaching the alveolar acini. These acini, comprised of parenchymal tissue, are highly susceptible to damage and are least protected by the bodies clearance mechanisms. Although, in the case of pyrotechnically generated aerosols the possible formation of combustion

gases must be considered. Combustion gases, such as carbon monoxide and carbon dioxide, are capable of altering respiratory function.

Data concerning the resulting toxicological effects of exposure to soluble, dry-powder aerosols are slowly becoming available. During the course of investigating the toxicity of several SFE formulations, the primary focus was on the aerosol part of the equation; what size was the aerosol, could it be inhaled and would it alter normal physiological parameters? However, satellite studies show that there was gaseous component to the equation; specifically elevated concentrations of carbon monoxide and carbon dioxide. Therefore, the objective of this study was to evaluate blood gases, hemoglobin, and blood pH of animals exposed to the by-product of SFE Formulation A1 and *A2,* as well as, to characterize their aerosol properties and combustion gas profiles.

Materials and Methods

Chemicals

SFE Formulation A1 and ***A2*** were supplied by Spectronix Ltd. (Tel Aviv, Israel). The general composition for SFE Formulation A1 and ***A2*** are listed in Table 1.

Table 1: General composition of SFE Formulation A1 and ***A2***

Components	SFE Formulation A1	SFE Formulation ***A2***
Oxidizer	68.0 %	72.0 %
Binder and Other	32.0 %	28.0 %
Total	100%	100%

Animals

Male Fisher CDF (F-344)/CrlBR rats were purchased from Charles River Breeding Labs (Wilmington, MA). The rats weighed between 200-250 g. The animals were provided Formula Lab Chow 5008 (Purina **Mills** Inc., St. Louis, MO) and reverse osmosis filtered water ***ad libitum.***

Experimental Design

Range Finding: Rats were randomized into groups of **4** animals and exposed to either air (control) or a SFE load of 50, 80, 140 or 240 g/m^3 for 15 or 60 minutes. Exposures were performed in a 700 L whole body inhalation chamber under static conditions. Blood and tissue samples were collected 1 hour post-exposure for blood gas analyses, hemotology, and histological examination. The generated aerosol atmospheres were characterized for mass aerosol concentration, mass median aerodynamic diameter (MMAD) and particle size distribution (σg). Exposures groups are as follows:

Exposure Group	SFE Load	Leneth of Exposure
Group 1	Control	60 min
Group 2	50 g/m^3	15 min
Group 3	80 g/m^3	15 min
Group 4	50 g/m^3	60 min
Group 5	80 g/m^3	60 min
Group 6	140 g/m^3	15 min
Group 7	240 g/m^3	15 min
Group 8	140 g/m^3	60 min
Group 9	240 g/m^3	60 min

Experimental Design

Multiple Dose: Rats were randomized into groups of 4 animals and exposed to either air (control) or a SFE load of 35, 50, or 80 g/m^3 for 15 or 60 minutes a day for *5* consecutive days. Exposures were performed in a 700 L whole body inhalation chamber under static conditions. Tissue samples were collected 1 hour post-exposure histological examination. The generated aerosol atmospheres were characterized for mass aerosol concentration, mass median aerodynamic diameter (MMAD) and particle size distribution (og).

Inhalation Chamber Configuration and Operation

The exposure system consisted of a modified Hinners-type 700 L inhalation chamber with a supply/exhaust system, a specially designed aerosol generator and an exhaust scrubber. The generator was connected to the inlet side of the system by a **3"** aluminum duct. The system was operated in the dynamic mode during the pyrolyzation of SFE until the generation of aerosol had ceased and the chamber was filled and at equilibrium. Control valves were installed in the exhaust, inlet and generator flow lines to transform the chamber from a dynamic to a static system. Exposures were conducted under static conditions. The test atmosphere generator consisted of two flanged 4" sections of schedule 80 stainless steel pipe bolted together. **An** 1/8" thick sintered stainless steel plate was located between the two sections of pipe. Air entered the generator through the lower plenum and passed through the sintered plate into the ignition plenum. The ignitor consisted of a ***6*** cm piece of 26 ga nichrome wire attached to insulated copper electrodes, and placed through the wall of the upper plenum. The nichrome wire was coiled to fit in the bottom of a ceramic combustion boat placed on the sintered plate. A current of 18 volt/6 amp was passed through the nichrome to produce a temperature of 550 to 600°C. Aerosol samples were collected from sampling ports located in the rear of the chamber. Samples were analyzed for concentration, particle size distribution (MMAD) and particle size analysis (og). The system was exhausted through a scrubber at the conclusion of each exposure.

Aerosol Concentration

The exposure aerosol mass concentration was determined using filter samples. Samples were collected on a 47 mm Gelman 61631 A/E glass fiber filter, that were stored in a decicator prior to use. Filters were weighed on a Cahn C-31 Microbalance (Fisher; Cincinnati, OH) and placed in a brass filter holder (IN-TOX Products, Albuquerque, **NM).** Samples were collected at 1, 5, 15, 30, 45, and 60 minutes, depending on the length of the exposure. The filter flow rate was 5 L/min. with a sampling time of 15 seconds.

Particle Size Distribution and Analysis

Mass weighted aerodynamic particle size distribution was determined using a cascade impactor (IN-TOX Products, Albuquerque, NM). The impactor designs were based on Marple's criteria (Marple, 1978). Aerosol particles were collected on 37 mm stainless steel substrates coated with apiezon grease to minimize particle bounce. A 47 mm Gelman 61631 A/E glass fiber filter was used as a final filter. Substrates and filter were weighed on the Cahn C-31 Microbalance or measured on a conductivity meter. Samples were collected at the beginning and end of each exposure. The impactor flow rate was 10 L/min with a sampling time of 4 to 15 sec. depending upon the time the sample was collected. Particle size was reported as the mass median aerodynamic diameter (MMAD) and particle size distribution as the standard geometic deviation (σg).

Gas Analyses

Oxygen was measured on a Teledyne Analytical Instrument Percent Oxygen Analyzer. Carbon dioxide and carbon monoxide were measured on a Beckman Industrial Model 865 Infared Analyzer. Samples were collected at 1, 5, 15, 30, 45, and 60 minutes, depending on the length of the exposure.

Clinical Observations, Postmortem and Blood Collection

Clinical signs were recorded during the exposure at 1, 5, 15, 30, 45 and 60 minutes, depending on length of the exposure. Animals, which were asigned to the range finding study, were euthanized within 30 minutes of exposure by intraperitoneal injection of a euthanasia mixture consisting of Ketamine HCI (Vetalor; Parke-Davis, Morris Plains, NJ) and Xylazine (Rompun; Mobay Corporation, Shawnee, KS). Once euthanized, the abdominal and thoracic cavities were opened and blood samples collected from the left ventricle of the heart with a 10 cc syringe containing heparin. A portion of each blood sample was transferred to a 3 mL Vacutainer containing heparin (green top) for serum chemistry, while the remaining portion was analyzed for blood gases and pH. Gross examination was performed on the trachea, lung, heart and abdominal organs. Animals, which were assigned to the multiple dose study, were euthanized at the conclusion of the five-day-exposure.

Blood Gas, pH, Hemoglobin and Serum Chemistry Analysis

Blood gases and pH were determined on a Ciba-Corning 288 Blood Gas Analyzer (Corning Diagnostics Corp., Medfied MA). Parameters analyzed were pH, partial pressure of oxygen and carbon dioxide and bicarbonate. Hemoglobin analysis was performed on a Ciba-Corning 2500 CO-oximeter (Corning Diagnostics Corp., Medfied, MA). Hemoglobin parameters were total hemoglobin, carboxyhemoglobin, methemoglobin, oxyhemoglobin and deoxyhemoglobin. Serum chemistries were performed on a Kodak Ektachem 700 Analyzer (Rochester, NY). Serum chemistry parameters were glucose, sodium, potassium, chloride, calcium, magnesium, and phosphorus.

Histopathology

After the gross examination was performed, the trachea and lungs were removed from the thoracic cavity and trimmed. To examine nasal turbinates, the head was removed and cut

transversely at the level of the incisive papilla and second palatal ridge using a Buehler Isomet low speed saw with diamond wafering blade (Evanston, IL). All tissue sections were placed in 10% neutral buffered formalin and decalcified for three days in 10% ethylenediaminetetraacetic acid (EDTA; Sigma, St. Louis, MO). The tissues were processed for histological examination (light microscopy). Each section was embedded in paraffin, sectioned at 3-4 microns, and stained with hematoxylin and eosin.

Statistical Analysis

A one factorial analysis of variance with Bonferroni Multiple Comparison was performed on all blood gases, pH, hemoglobin and serum chemistry parameters. The equality of variance was tested using Levene's test.

Results

Chamber Operation

A pressure pulse was noted 10-15 seconds after ignition and lasted for 5-10 seconds. Interior chamber temperature remained at ambient (22-26°C).

Arosol Mass Concentration (Range Finding)

SFE Formulation A1: The average initial aerosol concentration was 6.36, 10.12 and 17.01 g/m^3 for a SFE load of 50, 80 and 140 g/m^3, respectively. However, by the end of exposure period, the aerosol concentration was approximately 0.80 g/m^3 independent of SFE load. The aerosol half-life is 18.3, 14.4 and 13.2 minutes for a SFE load of 50, 80 and 140 g/m^3, respectively.

SFE Formulation A2: The average initial aerosol concentration was 5.56, 8.62, 12.33 and 17.06 g/m^3 for a SFE load of 50, 80, 140 and 240 g/m^3, respectively. However, by the end of exposure period, the aerosol concentration was approximately 0.76 g/m^3 independent of SFE load. The aerosol half-life is 20.3, 18.2, 16.7 and 12.0 minutes for a SFE load of 50, 80, 140 and 240 g/m^3, respectively.

Arosol Mass Concentration (Multiple Dose - SFE Formulation A2)

The average initial aerosol concentration was 4.47, 6.07 and 8.24 g/m^3 for a SFE load of 35, 50 and 80 g/m^3, respectively. However, by the end of exposure period, the aerosol concentration was approximately 0.67 g/m^3 independent of SFE load. The aerosol half-life is 23.2, 17.4 and 15.9 minutes for a SFE load of 50, 80 and 140 g/m^3, respectively.

Particle Size Analysis (Impactors - Range Finding)

SFE Formulation A1: The MMAD ranged from 1.94 to 2 54 pm, from 2.09 to 2.85 μm, and from 2.19 to 3.69 μm for a SFE load of 50, 80 and 140 g/m^3, respectively The og ranged from 1.6 to 1.7, from 1.6 to 1.7, and from 1.7 to 1.9 for a SFE load of 50, 80 and 140 g/m^3, respectively.

SFE Formulation A2: The MMAD ranged from 2.48 to 3.23 μm, from 2.73 to 3.82 μm, from 2.91 to 4.53 μm, and from 3.01 to 5.15 μm for a SFE load of 50, 80, 140 and 240 g/m^3,

SFE Load (g/m^3)	Carbon Dioxide (ppm)	Carbon Monoxide (ppm)	Oxygen (%)
50	10,530	2,658	18.5
80	9,731	6,675	18.3
140	**ND**	**ND**	**ND**
240	ND	**ND**	**ND**

(g/m^3)	(ppm)	(ppm)	(%)
50	14,240	0	20.8
80	21,500	0	20.6
140	37,700	0	20.8
240	60,800	0	20.4

Survivability (Range Finding)

SFE Formulation A1: The number of animals surviving each exposure are listed in Table 4. All animals were dead after a 60 minute exposure to a SFE load of 140 g/m^3. For a 15 minute exposure to a SFE load of 140 g/m^3, all animals survived. No further exposures were conducted.

SFE Formulation A2: The number of animals surviving each exposure are listed in Table 4. No deaths occurred during any of the exposure.

Survivability (Multiple Dose -- SFE Formulation AZ)

All animals survived the 5-day-exposure to SFE loads of 35, 50 and 80 g/m^3 for 15 and 60 minutes.

Table 4: Survivability of rats exposed to the by-products of SFE Formulation **AI** and ***A2*** (# o animals alive after exposure/# of animals in the group)

SFE Load (g/m^3)	SFE Formulation A1		SFE Formulation A2	
50	4/4	4/4	414	4/4
80	4/4	4/4	4/4	4/4
140	4/4	**0/4**	4/4	4/4
240	**ND**	**ND**	4/4	4/4

Clinical Observation

Animals exposed to SFE Formulation **A** exhibited signs of dypsnea, lack of coordination, lethargy, and coughinglsneezing. Head pulling or straining was observed frequently; that is, the animal would extend the head back, up and away from the body. **As** loads and length of exposure increased, these signs became more pronounced. Animals that survived appeared to recover once placed in fresh air.

Hemoglobin Analyses (Range Finding)

SFE Formulation A1: Hemoglobin analyses are shown in Figure 1. Total hemoglobin was within its biological range. Carboxyhemoglobin was significantly increased ($p<0.01$) in all exposure groups with the highest concentration observed in group **5.** Methemoglobin was the same for groups 2 and 3, increased in group 4 and significantly increased ($p<0.01$) in group **5.** Deoxyhemoglobin was decreased in groups 2 and 3, and significantly decreased ($P<0.01$) in groups 4 and **5.** Oxyhemoglobin was depressed in all exposure groups.

SFE Formulation A2: Hemoglobin analyses are shown in Figure 1. Total hemoglobin was within its biological range. Carboxyhemoglobin was significantly increased ($p<0.01$) for groups 8 and 9 (groups ***6*** through 9 not shown). Methemoglobin increased as the SFE load and exposure length increased. Deoxyhemoglobin was significantly decreased ($p<0.01$) in groups 8 and 9. Oxyhemoglobin was moderately elevated.

Blood Gas Analyses (Range Finding)

SFE Formulation A1: Blood gas analyses are shown in Figure 2. The pCO_2 increased for groups 2, 3 and 4 with increased loading and length of exposure, whereas group **5** ($80\ g/m^3$ for 60 minutes), had a level similar to that of the control group. The pO_2 decreased for all exposure groups with increased loading and length of exposure. Blood pH decreased for all exposure groups with increased load and length of **exposure** (Figure **3).**

*SFE Formulation **A2:*** Blood gas and pH analyses are shown in Figures 2 and **3.** There was no difference between the control group and exposure groups for pCO_2, pO_2, and pH

Histopathology (Range Finding and Multiple Dose)

No lesions were noted during gross and microscopic evaluation.

Discussion

Animals exposed to the by-products of SFE Formulation ***A2*** had a higher survivability rate than animals exposed to the by-products of SFE Formulations **A1.** Animals exposed to ***A2*** survived loads that were three times higher than the highest load survived by animals exposed to A1. The main clinical findings for animals exposed to the by-products of SFE Formulation A1 were altered hemoglobin levels, blood gas concentrations and whole blood pH. These same parameters were unaffected in animals exposed to the by-products of SFE Formulation A2.

The altered hemoglobin levels from animals exposed to A1 consisted of increased carboxy- and methemoglobin levels, and decreased oxy- and deoxyhemoglobin levels. These alterations were greatest in animals exposed to a SFE A1 load of **80** g/m^3 for 60 minutes. In addition pCO_2 was increased while pO_2 decreased as the SFE load and exposure length increased. The increase in pCO_2 would explain the observed decrease in whole blood pH. Overall, these data suggest impaired gas exchange capabilities, which resulted in a respiratory acidosis. Clinical observations of coughing and sneezing suggest an attempt to expel a noxious substance. Head pulling or straining may be interpreted as respiratory distress or discomfort. Thus, the formation of carboxyhemoglibin suggest the presence of carbon monoxide and the clinical signs of dypsnea, lack of coordination and lethargy suggest possible carbon monoxide intoxication.

Carbon monoxide (CO) is a colorless, odorless tasteless gas which is produced from the incomplete combustion of carbon-based material, It is rapidly absorbed when inhaled and does not elicit a coughing or sneezing reflex, a significant increase in ventilation, or sign of breathing difficulty. Gas data in Table **5** and 6 show that CO is formed during the pyrolyzation of **Al,** but not during the pyrolyzation of A2. The formation of CO during the pyrolyzation of formulation **AI** is most likely attributed to the non-stoichometric composition of the parent material and the presence of a carbon based binder.

Carboxyhemoglobin is formed when CO binds to hemoglobin. Hemoglobin transports oxygen throughout the body via the reversible binding between oxygen and the iron atom within the heme portion of the hemoglobin molecule. **Due** to its high binding affinity, CO once bound to hemoglobin, blocks the normal binding of oxygen with hemoglobin. Therefore, CO prevents the binding of oxygen to hemoglobin and interferes with the supply of oxygen to the tissues. This decrease in oxygen content is detected by peripheral chemoreceptors (carotid bodies), which through a negative-feedback-mechanism, attempt to correct the pO_2 imbalance by increasing respiration. This increase in respiration, in turns increases the amount of aerosol inhaled, thus increasing the possibility of pulmonary insult.

Carbon dioxide (CO_2) was also produced during the pyrolyzation of A1 and A2. CO, causes profound stimulation of ventilation, thereby, increasing the amount of inhaled aerosol by-products. The concentration of CO, in some exposures was as high as **10%.** At concentrations of **2%,** CO, will stimulate respiration. Simultaneously, high aerosol concentrations

will trigger reflex responses via stimulation of J receptors that slow respiration. Inhaling such an atmosphere could account for the clinical observation of dypsnea, coughing and sneezing.

Conclusion

The lack of carbon monoxide production during the pyrolyzation of ***A2*** appears to be the key difference in the survivability and toxicity of the two formulations. No differences were noted in the aerosols' physical characteristics, regardless of the formulation. Therefore, minimal toxic effects were observed in animals exposed to the by-products of SFE Formulation ***A2*** when compaired to the same biological parameters examined in animals exposed to the by-products of SFE Formulation **A1.** In addition, no deaths were reported after multiple exposures to SFE Formulation A2.

Acknowledgment/Disclaimer

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References

References available upon request.

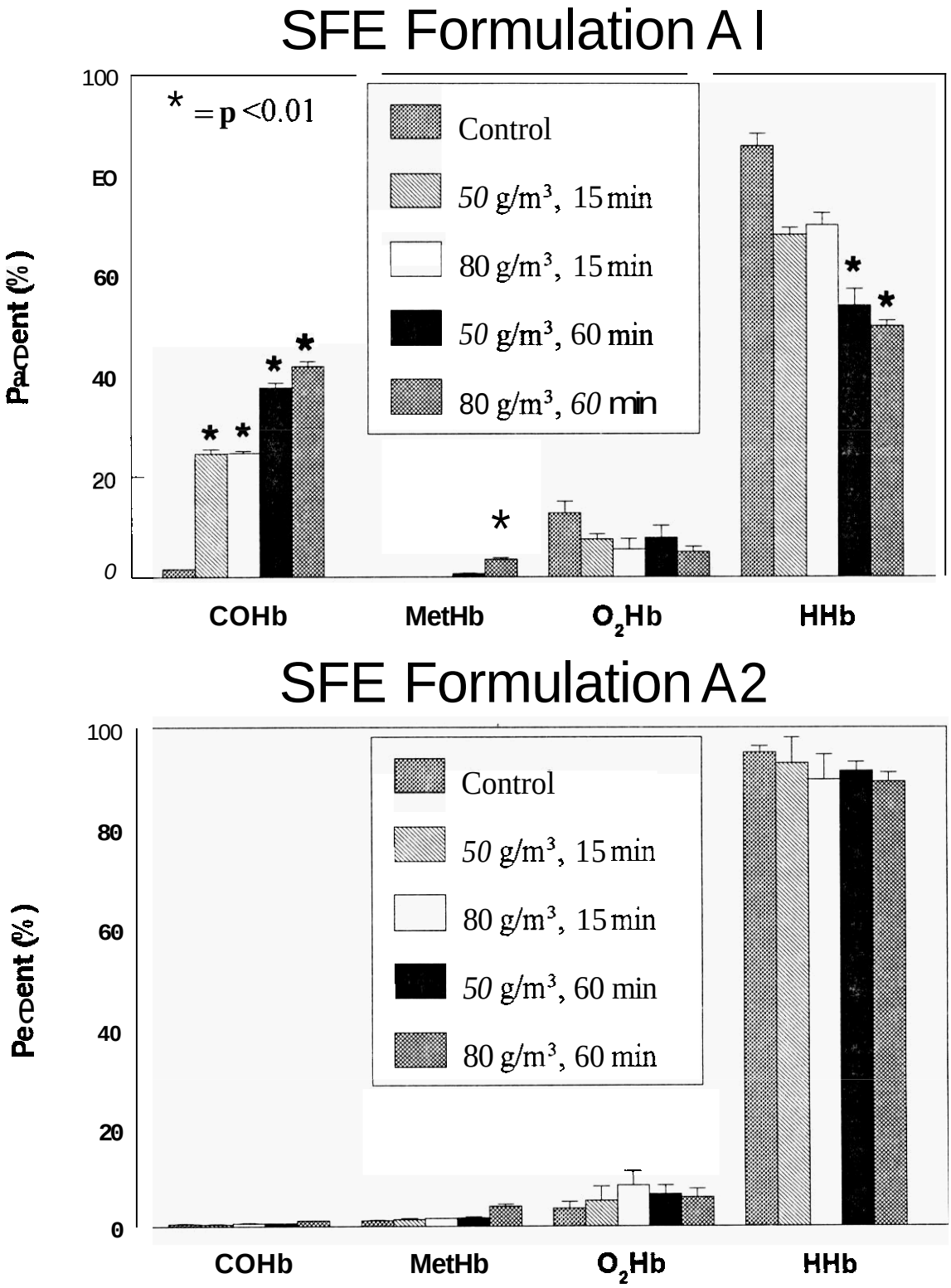

Figure 1. Hemoglobin analyses for rats exposed to the pyrolyzed by-products of SFE Formulation A1 and A2.

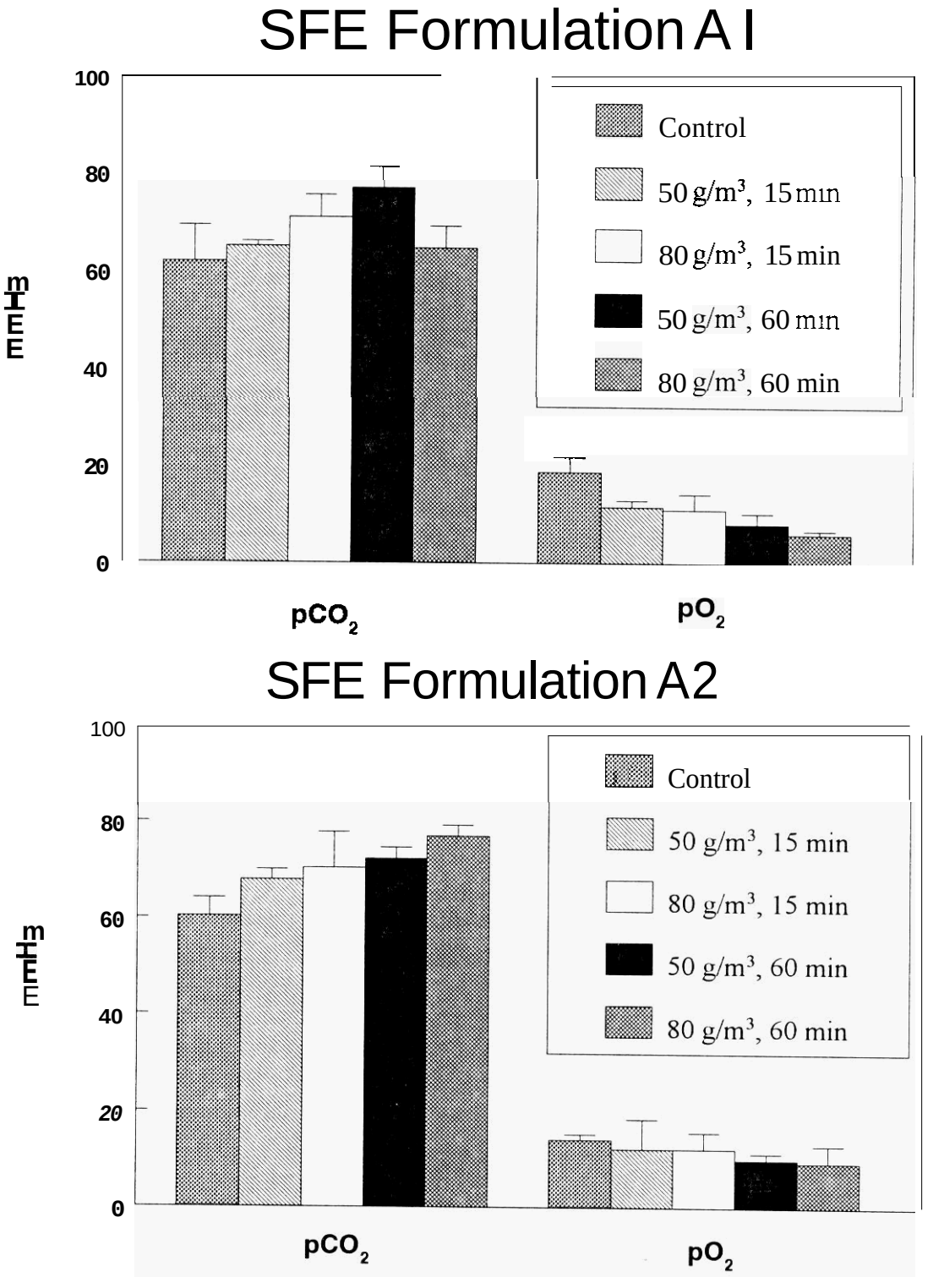

Figure **2.** Blood gas analyses for rats exposed to the pyrolyzed by-products of SFE Formulation A1 and A2.

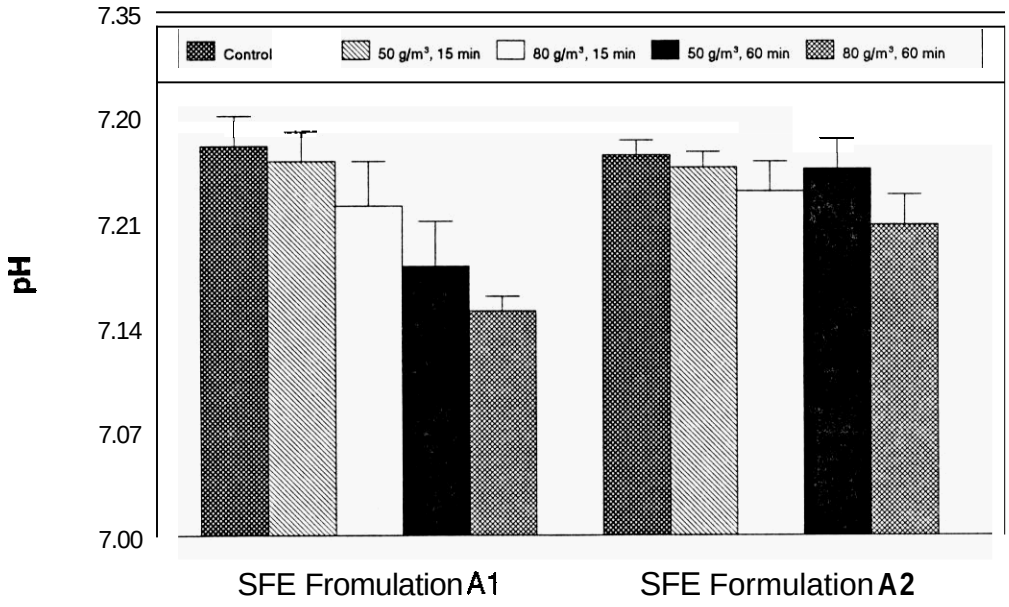

Figure 3. Blood pH analyses for rats exposed to the pyrolyzed by-products of SFE Formulation A1 and A2.